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Abstract*—**Increased dietary linoleic acid has been associated with reduced blood pressure in clinical and animal studies possibly mediated by prostaglandins. Urinary linoleate and prostaglandin metabolite excretion were investigated in subjects exposed to a salt-loading/salt-depletion regimen. Twelve healthy subjects were recruited from the New Orleans population (before Hurricaine Katrina) and admitted to the Tulane-Louisiana State University-Charity Hospital General Clinical Research Center after a 5-day outpatient lead-in phase on a 160-mmol sodium diet. On inpatient day 1, the subjects were maintained on the 160-mmol sodium diet, and a 24-hour urine specimen was collected. On day 2, the subjects received 2 L of IV normal saline over 4 hours and continued on a 160-mmol Na^+ diet (total: 460 mmol of sodium). Two 12-hour urine collections were obtained. On day 3, the subjects received three 40-mg oral doses of furosemide, two 12-hour urine collections were obtained, and the subjects were given a 10-mmol sodium diet. Urinary oxidized lipids were measured by high-performance liquid chromatography-tandem quadrupole mass spectroscopy. The excretion of the urinary linoleate metabolites, dihydroxyoctadecamonoenoic acids, and trihydroxyoctadecamonoenoic acids increased significantly during intravenous salt loading as compared with day 1 and the salt-depleted periods. The urinary excretion of 6-keto- prostaglandin F1α was unaffected by salt loading but was dramatically increased 7- to 10-fold by salt depletion. Prostaglandin E2 excretion was positively correlated with sodium excretion. The salt-stimulated production of linoleic acid diols and triols may inhibit tubular sodium reabsorption, thereby assisting in the excretion of the sodium load. (Hypertension*. 2008;51:755-761.)**

Key Words: linoleic acid ■ hypertension ■ salt loading ■ salt excretion ■ prostacyclin ■ PGE2

The dietary intake of linoleic acid (LA), an essential fatty acid, influences blood pressure. Increased dietary LA intake reduces systolic blood pressure, increases red blood cell membrane LA content, and alters red and white blood cell sodium transport processes in clinical studies.[1–7] Conversely, dietary LA deprivation in rats results in the development of salt-sensitive hypertension and an inability to excrete an acute salt load, with both effects reversed by the administration of LA.[8–10]

LA is a precursor of arachidonic acid, and it has been proposed that the effects of LA on blood pressure and salt excretion may be mediated through the production of various cytochrome P450 (CYP) eicosanoids and/or prostaglandins, including the epoxyeicosatrienoic acids (EETs)[11–14] and the prostacyclin metabolite 6-keto-prostaglandin (PG) F1α.[15,16] Inhibition of CYP-mediated EET production has been associated with the development of hypertension in rats.[11] Dietary salt loading upregulates CYP2C23 in rats, increasing EET production and inhibiting Na^+ reabsorption, whereas low-salt diets suppress this enzyme.[12,14] The EETs have also been shown to directly inhibit the distal tubule epithelial sodium channel.[13,14]

LA, however, also serves as a substrate in various oxygenation reactions. For instance, the direct metabolism of LA by the CYP epoxygenases (eg, CYP2C9 and CYP2J2) produces the 9(10)- and 12(13)-epoxyoctadeca(mono)enoic acids (EpOMEs), and hydration of these metabolites produces the corresponding 1,2- or vicinal dihydroxyoctadeca(mono)enoic

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acids (DiHOMEs), glucuronides of which are constitutive components of human urine.[17,18] It is particularly interesting that in vitro patch clamp studies show that LA and its metabolites suppress cardiac sodium channel function but do not stimulate amiloride-sensitive sodium currents, as the long chain omega-3 fatty acids can also do.[9,19–21] In human embryonic kidney cells expressing the α subunit of the human cardiac Na^+ channel, fatty acids appear to reduce sodium current in a dose- and voltage-dependent manner by binding to the inactivated state of the channel.[19] Moreover, EpOMEs and DiHOMEs have been shown to stabilize the inactivated state of the inward cardiac Na^+ current in isolated rat ventricular myocytes.[20]

Therefore, it must be considered that evidence arguing for linkage between CYP-dependent arachidonate metabolism and sodium handling may also apply directly to linoleate metabolism, with modulation of membrane ion transport in the kidney forming a plausible link between the physiological regulation of Na^+ balance and LA metabolism. To investigate this linkage, we conducted salt loading/depletion studies in human subjects and investigated the influence of this regimen on the urinary excretion of various oxygenated metabolites of linoleate and arachidonate. Here we report that the urinary excretion of LA diols and triols, and PGE2 is directly correlated with sodium excretion, whereas 6-keto-PGF1α excretion is inversely correlated with sodium excretion.

Methods

Salt Loading and Depletion Protocol

The salt-loading protocol was adapted from a previous protocol.[22,23] Twelve healthy subjects, ages 20 to 46 years, were recruited for the salt-loading and depletion protocol implemented in the Tulane-Louisiana State University-Charity Hospital General Clinical Research Center (GCRC) in New Orleans. Informed consent approved by the Tulane Institutional Review Board was obtained from all of the subjects. At this initial visit, 3 mL of blood was drawn to check their complete blood count. If their hemoglobin value was <10g/dL, they were not allowed to participate. The maximal allowable blood pressure (BP) during the entire 4-day inpatient GCRC study including the saline infusion period was 180/110 mm Hg.

Clinic Visit

The GCRC dietician met with the subjects in the GCRC outpatient clinic ≈7 days before admission to the GCRC inpatient unit to instruct the subject on compliance with the 160 mmol/d sodium diet that was followed for 5 days before admission. The dietary intake record obtained during the inpatient phase was analyzed for LA using the Nutrition Data Systems for Research software package 2006 version developed by the University of Minnesota Nutrition Coordinating Center. Only 7 of 12 subjects have complete inpatient dietary records because of the difficulty in accessing records at Charity Hospital closed after Hurricane Katrina.

Day 1 (Admission)

The healthy subjects were admitted to the GCRC and a physical examination, ECG, complete metabolic profile (7 mL of blood), and urinalysis were performed. A urine drug screen for drugs of abuse, the nicotine metabolite, cotinine, and urine pregnancy test were performed. If the drug screen, cotinine, or pregnancy tests were positive, the patient was excluded. The subject was weighed daily, and 24 hour urine collection (T1) was initiated at 8:00 AM for the measurement of sodium, potassium, creatinine, and a variety of oxygenated polyunsaturated fatty acids, including those derived from linoleate (9,10-EpOME; 12,13-EpOME; 9,10-dihydroxyoctadeca-[12*Z*]enoic acid [9,10-DiHOME]; 12,13-dihydroxy-octadeca[9*Z*]-enoic acid [12,13-DiHOME]; 9,10,13-tihydroxyocatdeca[12*E*]enoic acid [9,10,13-TriHOME]; 9,12,13-trihydroxyoctadeca[10*E*]enoic [9,12,13-TriHOME]; 9-hydroxy-octadecadienoic acid; and 13-hydroxyoctadecadienoic acid) and arachidonate (PGE2; PGD2; PGF2α; 6-keto-PGF1α; 8,9-EET; 11,12-EET; 14,15-EET; 5,6-dihydroxyeicosatrienoic acid [DHET]; 8,9-DHET; 11,12-DHET; 14,15-DHET; and 20-hydroxyeicosatetraeinoic acid) and kept on ice. Thirty milliliters of blood was drawn for plasma renin, aldosterone, and basic chemistry at the midpoint of the collection at 8 PM. The subject continued to adhere to a 160-mmol/d sodium diet.

Day 2 (Salt Loading)

The subject continued to adhere to a 160-mmol/d sodium diet. Before 8:00 AM the subject was fitted with a 24-hour ambulatory BP monitoring Oscar-2 device (Sun Tech Medical Inc) programmed to determine BP every 15 to 30 minutes (15 minutes while awake and 30 minutes while sleeping), and plasma renin, aldosterone, and a basic chemistry were determined. At 8:00 AM, the subject was given 2 L of normal (0.9%) saline IV over 4 hours, and a 12-hour urine specimen was collected from the start of the infusion and continued until 8:00 PM (T2). At 8:00 PM, 30 mL of blood was drawn for the measurement of plasma renin, aldosterone, and a basic chemistry profile determination, and a second 12-hour urine collection was started (T3 start). Aliquots of urine from both 12-hour collections were taken and frozen for lipid metabolite, sodium, potassium, and creatinine analyses.

Day 3 (Salt Depletion)

The second 12-hour urine collection was completed at 8:00 AM (T3 end), and 30 mL of blood was drawn as before for renin, aldosterone, and basic chemistry profiling. The subject was placed on a 10-mmol/d sodium diet and given 40 mg of oral furosemide at 8 AM, 12 PM, and 4 PM. At 8 PM a third 12-hour urine collection was initiated (T4 start) for the measurement of the same analytes. The average BPs from 12:00 PM to 10:00 PM on days 2 and 3 were used for classification of salt resistant and salt sensitive. A fall of 10 mm Hg systolic BP from day 2 to day 3 was the cutoff to define salt sensitivity.[22]

Day 4 (Termination)

The 24-hour BP arm cuff and monitor were discontinued before 8 AM. The subject was maintained on a 10-mmol/d sodium diet. At 8 AM, the third urine collection was completed (T4 end), and 30 mL of blood was drawn for the same plasma analytes. At that time, the fourth 12-hour urine collection was initiated (T5 start). At 8 PM the fourth urine collection was completed (T5 end), and 30 mL of blood was again drawn for the same analytes. The subject was then discharged from the GCRC.

Isolation and Quantification of Urinary Oxygenated Lipids

All of the chemicals and reagents used were purchased from σ-Aldrich and used without further purification unless otherwise specified. Linoleate diols were purchased from Cayman Chemical, whereas linoleate triols were purchased from Larodan Fine Chemicals. The internal standard 1-cyclohexyl-3-docosahexanoic acid urea and diol surrogate 10,11-dihydroxynonadecanoic acid were synthesized and purified as described previously.[18]

Oxygenated lipids were extracted using solid phase extraction techniques. Urine samples were removed from the −80°C freezer and allowed to thaw to room temperature. A 2-mL sample aliquot was spiked with a 10-μL aliquot of 0.2 mg/mL EDTA/borderline hypertension in methanol:water (1:1). An equivalent aliquot of 0.1 mmol/L of sodium phosphate (pH 7.4) served as a procedural blank and was spiked in an identical manner. Spiked samples were then subjected to glucuronidase treatment before extraction. Glucuronidase treatment was performed as follows: 500 μL (200 U) of *Helix pomatia* type H1-glucuronidase (Sigma) in 1 mol/L of sodium citrate (pH 5.0) was added to each sample (including Blank and Matrix Spike) and allowed to incubate for 4.5 hours in a 37°C water bath. Solid phase extraction cartridges (60 mg Oasis HLB, Waters

Table 1. Subject Characteristics

Subject	Age, y	Weight, kg	Gender*	Ethnicity†
1	41	99.6	M	B
2	26	73.5	F	W
3	24	95.8	M	B
4	28	74.6	M	B
5	31	76.9	F	B
6	42	74.1	M	W
7	46	84.5	M	B
8	28	77.7	F	B
9	31	57.0	F	B
10	23	74.1	F	B
11	37	63.5	F	B
12	20	70.8	M	B
Mean±SD	31.4±8.3	76.8±12.1		

*Female (F):male (M) subject distribution was 6:6.
†Black (B):white (W) subject distribution was 10:2.

Inc) were cleaned with 5 mL of methanol and conditioned with 5 mL of 5:95 methanol/water (vol/vol) acidified with 0.1% acetic acid. Samples were loaded to the solid phase extraction cartridge reservoir, immediately spiked with analytical surrogates (final concentration: 100 nM) in 10 μL of methanol, and eluted through the column by gravity. Loaded cartridges were washed with 10 mL of 0.1% acetic acid in water followed by 4 mL of 0.1% acetic acid and 5% methanol by gravity. Cartridges were dried using low vacuum (<20 KPa). Dried cartridges were eluted with 2 mL of ethyl acetate and blown down just to dryness using a gentle stream of nitrogen gas. Samples were reconstituted in 50 μL of methanol containing the internal standard solution, slightly vortexed, and transferred to inserts for analysis.

The oxygenated lipids in 20-μL extract aliquots were separated by reverse-phase high-performance liquid chromatography on a 2.1×150 mm, 5 μmol/L Luna C18[2] column (Phenomenex) and quantified using a Quattro Ultima tandem quadrupole mass spectrometer (Micromass) with negative mode electrospray ionization and multiple reaction monitoring, as described previously.[24] The relative response ratios of analytes were used to calculate analyte concentrations while correcting for surrogate losses. Surrogate recoveries were evaluated by quantification against the internal standard. The recovery of 6-keto-PGF1α was corrected for the recoveries of its tetra-deuterated analog (6-keto-PGF1α-d4). Recoveries of 6-keto-PGF1α-d4 were 15±5% using the described extraction protocol. Although these surrogate recoveries are low for this polar metabolite, they were consistent across all of the experimental periods and random for all of the analyzed samples, such that a systematic bias in reporting was not apparent. All of the other reported analytes were corrected for 10,11-dihydroxynonadecanoic acid recoveries, which were 65±7%. Blanks showed less than 3 times the detection limit for all of the reported residues. Replicate precision was acceptable: ie, ≥75% of the analytes with concentrations greater than the detection limit had precision ≤20%. The metabolite levels were expressed as nanomoles per mole of creatinine per hour. The hydroxyoctadecadienoic acid EpOME, hydroxyeicosatetraeinoic acid EET, and DHET metabolites were measured, but values were uniformly near or below the level of quantitation for the assay such that assessments of relationships with time and Na^+ excretion had little power and are not reported.

Statistics

We computed means and SDs for all of the variables. Highly skewed data were log transformed before statistical evaluations. To reduce interindividual variability, time course data were normalized to the range of excreted values observed for each individual using equation 1:

$$(1) \qquad \frac{X-\min(T_1, \ldots, T_5)}{\max(T_1, \ldots, T_5)-\min(T_1, \ldots, T_5)}$$

where the oxygenated lipid concentrations are $X=(T_1, \ldots, T_5)$. Both raw and normalized results for each metabolite were analyzed with repeated-measures 1-way ANOVAs and Neuman-Keuls posthoc tests to describe differences using GraphPad Prism version 4.03. We used linear regression to estimate the correlation between sodium concentration and each of the oxygenated lipids. Regression analyses of longitudinal results were evaluated for significance using a fixed-effects model, Proc Mixed, with auto regression as selected by AI and BI criterion after application of the MIVQUE0 methods in SAS version 6.14.

Results

Subject Characteristics and Dietary Linoleate Intake

The characteristics of the 12 healthy subjects are shown in Table 1. The majority of the subjects were blacks with an equal number of male and females. Three subjects were salt sensitive as defined by Grim et al.[22] Exclusion of these subjects did not alter the results. Assessment of dietary records indicated that LA intake during the inpatient stay was 17.1±4.4 g on day 1, 11.2±4.6 g on day 2, 12.5±0.5 g on day 3, and 12.5±0.5 g on day 4. The intake on day 1 (on 160-mmol sodium diet) is significantly greater than day 2

Table 2. Time Course of Urinary Linoleate Diols and Triols Excretion

Time Period	T1	T2	T3	T4	T5
Experimental day	1.0 to 2.0	2.0 to 2.5	2.5 to 3.0	3.5 to 4.0	4.0 to 4.5
Treatment	High salt	High salt IV	High salt	Low salt	Low salt
9,10-DiHOME, mean±SD, nmol/mol of creatinine/h	585±385	1983±1233*	1614±1072*	655±505	953±808
12,13-DiHOME, mean±SD, nmol/mol of creatinine/h	43.4±32.3	153±120*	101±66.3*	19.9±20.1†	29.8±24.4
9,10,13-TriHOME, mean±SD, nmol/mol of creatinine/h	26.4±17.5	75.7±39.5*	70.6±35.0*	25.7±22.5	21.5±22.2
9,12,13-TriHOME, mean±SD, nmol/mol of creatinine/h	314±318‡	998±1069‡	559±592‡	120±252	55.7±48.8

Results are the mean±SD; nmol/mol creatinine/h.
*$P<0.05$, T2 and T3>T1, T4, and T5; †$P<0.05$, T1>T4; ‡$P<0.05$, T1, T2, and T3>T4 and T5; T2>T1.

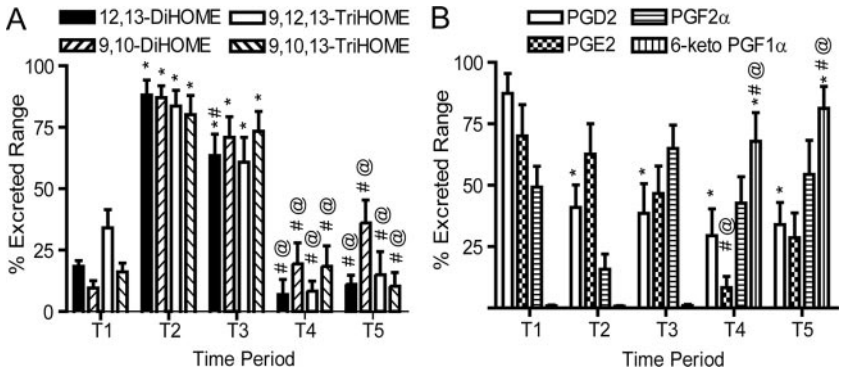

Figure 1. Urinary linoleate (A) and PG (B) metabolite excretion patterns associated with sodium loading and depletion. Metabolite concentrations observed during the experimental course for each subject were normalized to the individuals range in observed excretion using equation 1 (see text). The normalized results were analyzed with repeated-measures 1-way ANOVAs and Neuman-Kuels posthoc tests to describe differences using GraphPad Prism version 4.03. Differences are indicated at $P<0.01$ for the following comparisons: *vs T1, #vs T2, and @vs T3. All of the results are displayed as the calculated means±SDs of 12 subjects for all of the metabolites except PGE2 (n=10) and 6-keto-PGF1α (n=9).

(460-mmol sodium diet+IV saline) and days 3 and 4 (10 mmol of sodium).

Oxygenated Lipid Excretion

The time course of urinary linoleate metabolic excretion is shown in Table 2. There was a statistically significant 3- to 4-fold increase in the urinary excretion of 9,10-DiHOME, 12,13-DiHOME, 9,10,13-TriHOME, and 9,12,13-TriHOME during the day of intravenous salt loading (day 2.0) compared with day 1.0 baseline, which then dropped toward baseline during salt depletion on day 3.5 and day 4.0. Specifically, the 9,10-DiHOME excretion rose during the saline infusion and was maintained for 24 hours and then dropped back to baseline during the salt deprivation on days 3.5 and 4.0. The 12,13-DiHOME excretion follows a similar pattern; however, the excretion drops below baseline on day 3.5 and returns to baseline by day 4.0. The 9,10,13-TriHOME follows an identical pattern to the 9,10-DiHOME. The 9,12,13-TriHOME excretion increases immediately during the saline infusion but falls back to baseline after the end of the saline infusion on day 2.5 to 3.0, and then it drops below baseline during the salt-deprivation phase. Normalizing the results to the fraction of the range observed for each patient as shown in Figure 1A reduced the variance in the data but did not create the observed trends.

Changes in urinary PG excretion were also observed during this study, as shown normalized to the fraction of the range observed for each patient in Figure 1B. Levels of PGD2 dropped from day 1 baseline and remained constant for the remainder of the study, whereas changes in PGF2α were not detected. Conversely, the excretion of PGE2 was significantly reduced on day 4. In addition, in the 9 subjects for which data were available, the prostacyclin metabolite 6-keto-PGF1α did not change with salt loading on days 1 and 2 but rose 7- to 10-fold during salt depletion on days 3 and 4 (Figure 1B). Urinary levels of other measured eicosanoids and octadecanoids, including the epoxy fatty acids and 20-hydroxyeicosatetraeinoic acid, were below limits of quantification, precluding evaluation of their behavior with respect to the described experimental parameters.

Sodium Excretion

Sodium excretion immediately rose from baseline during the saline infusion, returned to baseline by the end of the infusion, and dropped below baseline after 3 oral doses of furosemide. The Na^+ excretion time course is shown in Figure 2.

Sodium/Oxygenated Lipid Interactions

There were significant correlations ($P<0.001$) between the urinary excretion of sodium and LA diols and triols and sodium excretion (9,10-DiHOME, $r=0.469$; 12,13-DiHOME, $r=0.763$; 9,10,13-TriHOME, $r=0.625$; and 9,12,13-TriHOME, $r=0.636$). The renin and aldosterone levels (n=9) showed a significant stimulation during salt depletion on days 3 and 4 (Figure 3), which were positively correlated with each other and with urinary 6-keto-PGF1α and negatively correlated with the urinary Na^+, LA diols, and triols (Figure 4). Consistent with these findings, the Na^+ and 6-keto-PGF1α excretions were also inversely correlated (Figure 5A). The urinary excretion of PGE2 was positively correlated with sodium excretion (Figure 5B).

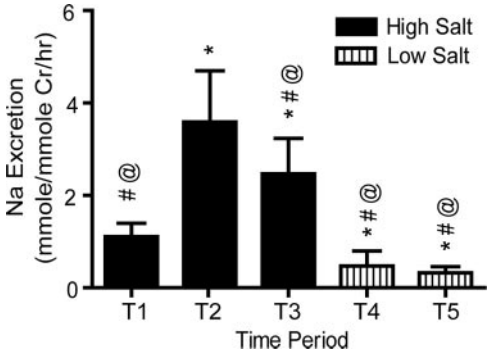

Figure 2. Time course of urinary sodium excretion. Significant increases of Na excretion during the intravenous salt loading on day 2 and below baseline on day 3 and day 4 are indicated, with all P values <0.05: *vs T1, #vs T2, and @vs T3. All of the results are displayed as the calculated means±SDs of 12 subjects.

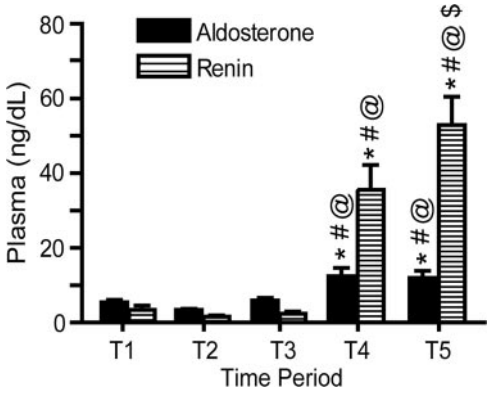

Figure 3. Changes in plasma aldosterone and renin associated with sodium loading and depletion. Results for each metabolite were analyzed with repeated-measures 1-way ANOVAs and Neuman-Kuels posthoc tests to describe differences using GraphPad Prism version 4.03. Differences are indicated at $P<0.01$ for the following comparisons: *vs T1, #vs T2, @vs T3, and $vs T4. All of the results are displayed as the calculated means±SDs of 9 subjects.

Discussion

Several previous findings have suggested a link between sodium metabolism and LA. Clinical studies have shown that an increase in dietary intake of LA inhibits red and white blood cell Na^+/Li^+ countertransport and reduces BP.[1–7,10] In addition, in vitro studies have demonstrated that LA metabolites inhibit cardiac sodium channels.[19,20] Several studies in rats have shown the development of salt-sensitive hypertension with the inability to excrete an acute salt load after dietary LA acid deprivation that is corrected by administration of LA, suggesting that LA is intimately involved with renal sodium handling.[8,9] The results we report here reveal an increase in urinary excretion of LA diols and triols during intravenous salt loading that correlate with sodium excretion. This may be because of increased renal production of these metabolites, with the increased urinary LA metabolite excretion reflecting a compensatory mechanism assisting in the excretion of a salt load by inhibiting the reabsorption of sodium along the nephron, as in the rat LA deprivation studies.

Dietary logs suggested a significantly increased LA intake on day 1 compared with day 2, with no differences between days 2, 3, and 4. It is possible that the drop in dietary intake may be contributing to the drop in urinary excretion of the LA diols and triols; however, the peak in urinary excretion was not on day 1 but on day 2 during the saline infusion with a decline on day 3 and day 4. A recent study investigating the effect of acute changes in dietary intake on urine metabolites showed rapid changes in urine metabolite profile within 24 hours of starting a standardized diet.[25] The changes were seen by the next morning. This cannot explain the 3-fold rise in LA diols and triols throughout day 2.

The direct renal tubular effects LA metabolites have not been extensively studied. Such effects may be mediated through increased production of cyclooxygenase-derived prostanoids and/or CYP eicosanoids. In the cited studies of LA deprivation, urinary PGE2 excretion was inhibited, which could explain the effects on sodium excretion.[8,9] Intrarenal infusion of PGE2 is known to increase renal Na^+ excretion more potently than the prostacyclin analog iloprost.[26] The decline in PGE2 detected here (0.5±0.2 to 0.2±0.1 nM from day 1 to day 4) and positive correlation with Na excretion was significant and expected, considering its potent natriuretic effects, and PGE2 probably plays a mechanistic role in the observed natriuresis. The 7- to 10-fold increase in the urinary prostacyclin metabolite 6-keto-PF1α level during salt depletion is probably in response because of the volume depletion after furosemide. Previous human salt loading and salt depletion studies have shown mixed results with regards to prostacyclin metabolism, with elevated 6-keto-PGF1α excretion occurring with both salt loading and depletion.[16,27] In the current study, this metabolite remained unchanged during the initial salt-loading phase and was negatively correlated with sodium excretion. Prostacyclin has been shown to cause afferent arterial vasodilation and stimulates renin release with resulting increases in angiotensin II and aldosterone during volume depletion leading to sodium reabsorption.[28] This mechanistic association is consistent with the negative correlation observed between urinary 6-keto-PGF1α and Na^+ excretion.

Renal sodium balance handling is also known to be regulated through the production of CYP metabolites of arachidonic acid, including the epoxy fatty acids (ie, EETs). The EETs inhibit proximal tubule sodium reabsorption by reducing the residence time of the Na^+/H^+ exchanger in the apical membrane and directly inhibiting the epithelial sodium channel.[11–14] Low-sodium diets reduce the expression of rat

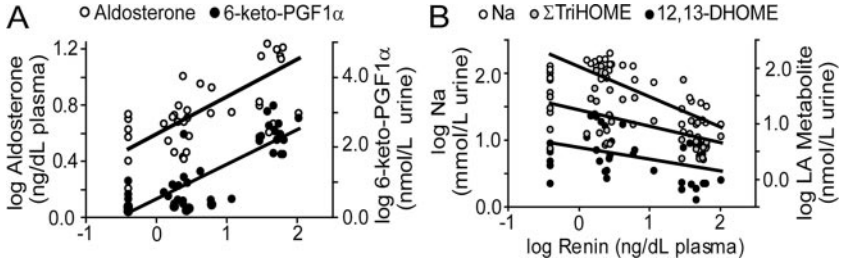

Figure 4. Regression analyses of plasma aldosterone and various urinary lipids with plasma renin concentrations. Significant positive regressions (A) were observed for aldosterone ($y=0.27x+0.59$; $r^2=0.52$) and 6-keto-PGF1α ($y=0.99x+0.54$; $r^2=0.67$). Significant negative correlations (B) were observed with sodium ($y=-0.46x+2.11$; $r^2=0.66$) and the sum of the urinary linoleate triols ($y=-0.30x+1.25$; $r^2=0.24$) but not the 12,13-DHOME ($y=-0.21x+0.57$; $r^2=0.15$). Regression analyses of longitudinal results were evaluated for significance using Proc Mixed with auto regression as selected by AI and BI criterion after application of the MIVQUE0 methods in SAS version 6.14.

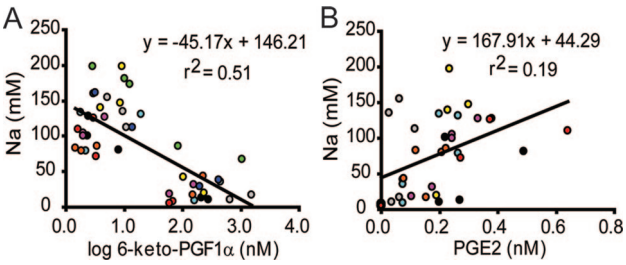

Figure 5. Urinary sodium was correlated with 6-keto-PGF1a (n=9; $P<0.001$) and PGE2 (n=7; $P<0.01$) excretion. Colored data points indicate single experimental subjects, whereas the line represents the regression analysis of the total data set. Regression analyses of longitudinal results were evaluated for significance using Proc Mixed with auto regression as selected by AI and BI criterion after application of the MIVQUE0 methods in SAS version 6.14.

CYPC23, reducing EET production and increasing sodium reabsorption.[14] This is suggested by the work of Yu et al,[29] which demonstrated an induction of CYP2C and 2J isozymes and increased renal cortical EETs in rats fed borage oil diets, which are high in γ-linolenic acids. The γ-linolenic acid can be converted to arachidonic acid by chain elongation and desaturation steps as LA. Although cardiac and epithelial sodium channels are structurally unrelated, the data on cardiac sodium channels might point toward the distal tubule epithelial sodium channel as a possible target for inhibition by LA diols and triols.[19,20] Although the biological role and synthesis of LA-derived triols are uncharacterized in mammals, these metabolites are present in the urine[17] and are likely generated by the enzymatic rearrangement of linoleate hydroperoxides.[30]

Other relevant findings suggesting associations between octadecanoids and hypertension have been reported recently. In clinical studies, plasma levels of the LA metabolite 12(13)epoxy-9-keto-octadeca(10*E*)enoic acid correlated with systolic hypertension and obesity in blacks.[31] In rat adrenal cells, 12(13)epoxy-9-keto-octadeca(10*E*)enoic acid has been shown to be positively correlated with aldosterone production under conditions of submaximal effects of potassium and angiotensin II. The urinary LA diols and triols measured here, however, are negatively correlated with plasma aldosterone. Levels were high during the salt-depletion phase with accompanied volume depletion because of furosemide, as expected.

A variety of other effects are also known. Recent studies have shown that LA and its metabolites exhibit proinflammatory effects in the vascular endothelium and cytotoxicity to proximal tubule cells, such that LA may be associated with some adverse effects as well.[29,32–34] Some cell-based toxicity studies have also shown a protective effect of LA but not its diols.[35] The monoepoxides of LA, leukotoxin and isoleukotoxin, have been associated with multiorgan failure,[32] which has been linked to the production of LA diols in proximal tubule cells.[35] Moreover, structure/function evaluations suggest that 1,2-diols are structurally optimized to elicit soluble epoxide hydrolase-dependent biological effects.[36] These findings, together with the current study, supports the contention that LA metabolites have potent effects in renal tissues and suggest that the concentration at the site of action likely determines whether the effects are beneficial or not.

Perspectives

Little is known about the renal and cardiovascular effects of the abundant LA metabolites. Our results show increased urinary excretion of LA diols and triols during intravenous salt loading, which correlate with sodium excretion. We also demonstrate dramatic increases in the urinary excretion of 6-keto-PGF1α later during salt depletion and positive correlation of PGE2 excretion with salt excretion. These results suggest that LA metabolites may have direct tubular effects on renal sodium handling or may exert their effects indirectly through the generation of eicosanoids, thus aiding in excretion of a salt load. Further study is warranted to elucidate the mechanisms by which LA metabolites affect renal sodium handling and hypertension.

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Disclosures

None.

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